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TEPOXALIN, A NOVEL IMMUNOMODULATORY COMPOUND, SYNERGIZES WITH CSA IN SUPPRESSION OF GRAFT-VERSUS-HOST REACTION AND ALLOGENEIC SKIN GRAFT REJECTION

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Tepoxalin, a dual 5-lipoxygenase and cyclooxygenase inhibitor with nonsteroidal antiinflammatory effects, has recently been shown to suppress NFκB transactivation and inhibit T cell proliferation via a mechanism very different from cyclosporine (CsA). In this report, we demonstrate that this novel immunosuppressive effect of tepoxalin is manifested in *in vivo* transplantation models. Tepoxalin suppressed murine spleen cell proliferation in a mixed lymphocyte reaction (MLR) with an IC_{50} of 1.3 μM. Coadministration of tepoxalin and CsA in MLR cultures showed an additive inhibitory effect. Oral administration of tepoxalin at 12 mg/kg/day to mice suppressed local graft-versus-host (GVH) responses by about 40% (n=10). Combination of tepoxalin and CsA at suboptimal doses synergized their immunosuppressive effects on GVH responses (n=20). In skin transplantation, the median survival time of allogeneic BALB/cByJ (H-2^d) mouse skin grafted onto C3H/HeJ (H-2^k) mice was 10.5 days (n=8), and was prolonged to 15.0 days (n=9) for recipient mice administered tepoxalin at 50 mg/kg/day. Coadministration of suboptimal doses of tepoxalin (12.5 mg/kg/day) and CsA (50 mg/kg/day) prolonged skin graft rejections dramatically (55% of the grafts survived for more than 40 days, n=9). Taken together, these results demonstrate that tepoxalin is a potent immunomodulatory compound that, when combined with CsA, provides synergistic immunosuppressive activity. The fact that tepoxalin and CsA act on different transcription factors, NFκB and NFAT respectively, might explain the synergistic suppressive effects when both compounds were used. Tepoxalin could be an important addition to the cohort of immunosuppressive therapies currently used in solid organ and bone marrow transplantations.

The immune response in transplantation, which results in graft rejection and graft-versus-host (GVH)[*] response, is primarily triggered by T cells through recognition of alloantigens (*1–4*). Suppression of immune response could be achieved using agents interfering with T cell activation and effector functions. The use of cyclosporine (CsA) as an immunosuppressant in transplantation has been documented (*5, 6*). CsA inhibits T cell activation by inhibiting the nuclear translocation of the nuclear factor NFAT (*7, 8*). However, CsA has associated toxicities and side effects when used at therapeutic doses (*9*). Compounds that suppress T cell–mediated immune response with mechanisms different from that of CsA will undoubtedly be valuable additions to the cohort of the current regiments.

Tepoxalin (*5*-[4-chlorophenyl]-*N*-hydroxy-[4-methoxyphenyl]-*N*-methyl-1-*H*-pyrazole-3-propanamide) was discovered originally as a dual inhibitor of 5-lipoxygenase (LO) and cyclooxygenase (CO) and exhibits potent nonsteroidal antiinflammatory activities in animal models of adjuvant arthritis (*10–12*). Recently we found that tepoxalin also inhibits OKT3-induced T cell proliferation via a mechanism very different from that of CsA (*13*). CsA is known to block IL-2 production after activation of T cells through TCR/CD3, whereas tepoxalin inhibits IL-2 induced signal transduction (*13*). An in-depth investigation of the mechanism of action reveals that tepoxalin inhibits predominantly NFκB activation (*14*), whereas CsA is most effective in blocking NFAT transactivation (*7, 8*). Because of these different mechanism of actions, a possible additive/synergistic effect of the combined tepoxalin and CsA treatment is expected. In this report, we demonstrate that tepoxalin is indeed effective in suppressing mixed lymphocyte reactions (MLR), GVH responses, and allogeneic skin graft rejections in mice. The synergistic effect of tepoxalin and CsA in immunosuppression was also studied. The possible mechanism of tepoxalin in immunosuppression and its potential clinical application are discussed.

MATERIALS AND METHODS

Mice. Inbred C57BL/6J, C3H/HeJ, and BALB/cByJ mice and B6D2F$_1$/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Those used in experiments were male mice at about 6–10 weeks of age that weighed 18–25 gs.

Preparation of test compounds. Tepoxalin, naproxen, and zileuton were synthesized by the R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ). CsA (Sandimmune i.v.) was from Sandoz (Quebec, Canada). For MLR experiments, stock solutions of tepoxalin, naproxen, and zileuton were prepared in DMSO at 30 mM and diluted to working concentrations in culture medium at the time of experiments. DMSO at concentrations equivalent to those of the test compounds were used as controls in MLR assays. For experiments of GVH responses and skin graft rejections, micronized tepoxalin and naproxen were suspended in 0.5% methylcellulose (Sigma, St. Louis, MO) at concentrations of 5 mg/ml or lower. The vehicle control was the equivalent volume of 0.5% methylcellulose. Zileuton was dissolved in 50% polyethylene glycol 200 (Sigma, St. Louis, MO), and the corresponding vehicle control was the equivalent volume of polyethylene glycol 200. CsA was diluted in saline. All compounds were dissolved in vehicle just prior to administration to mice at volumes of

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[*] Abbreviations: CsA, cyclosporine; CO, cyclooxygenase; GVH, graft-versus-host; LO, 5-lipoxygenase; MLR, mixed lymphocyte re-

Lymphocyte proliferation assays. Single-cell suspensions from mouse spleens were washed once with PBS and then resuspended in RPMI 1640 medium supplemented with 5% fetal bovine serum and 50 μM 2-mercaptoethanol. Responder spleen cells (2.5×10^5) from C57BL/6J mice (H-2^b) were stimulated by 2.5×10^5 irradiated (2000 rads) spleen cells from B6D2F_1/J mice (H-$2^{b/d}$). The responder and the stimulator cells were cocultured in 250 μl medium containing various concentrations of the tested compounds in the 96-well plates (round bottom wells, Corning Inc., NY). After 5 days of stimulation, ^{3}H-thymidine was added to the cultures (0.5 μCi per well) for 4 hr. Plates were harvested using a Tomtec Harvester 96, MACH II (Tomtec Inc., Orrange, CT) and samples were counted using a Wallac 1205 Betaplate scintillation counter (Pharmacia, Uppsala, Sweden).

Cell viability test. Cell viability was assessed with the MTT assay. Spleen cells from C57BL/6J mice were prepared in RPMI 1640 medium supplemented with 5% fetal bovine serum and 50 μM 2-mercaptoethanol. Spleen cells (2×10^5/well) were stimulated with immobilized anti-CD3 (Pharmingen) in the presence of tepoxalin or its vehicle DMSO in 96-well culture plates (Corning Inc., NY). The MTT assay was conducted by using the Celltiter 96 kit (Promega Corp.), which is based on the conversion of a tetrazolium salt by viable cells into a detectable blue formazan.

Graft-versus-host reactions. The GVH assay was based on the method of Dorsch and Roser (*15*). Spleen cells from C57BL/6J mice were injected subcutaneously into the footpads of B6D2F_1/J mice. Each footpad was injected with 8×10^6 spleen cells in 50 μl. Seven days later, the draining popliteal lymph nodes were removed, trimmed of fat and weighed. Mice injected in the footpads with saline were used as negative controls. Lymph nodes of these mice were indistinguishable from those injected with syngeneic spleen cells. Tepoxalin was administered orally and CsA was given subcutaneously to mice daily started one day before footpad injection unless otherwise specified.

Skin graft transplantation. C3H/HeJ mice (H-2^k) were anesthetized by intraperitoneal injection of 2.5% avertin (0.016 ml/g body weight). A grafting bed (about 0.3 cm×1 cm) on the mouse tail was prepared by peeling off skin carefully to avoid bleeding. Tail skin of similar size was peeled from BALB/cByJ mice (H-2^d) and then placed over the graft site in an opposite orientation according to the hair growth direction. The grafted skin was protected by a plastic tubing (diameter 0.5 cm, length 3 cm) held in place by wound clips for 5 days. Skin grafts were examined and scored daily. A graft was scored as being rejected when more than 80% of the graft was necrotic. CsA was given subcutaneously to mice daily starting one day before skin transplantation until rejection of grafts. Tepoxalin was given orally one day before transplantation and then daily starting one day after transplantation until graft rejection.

Data presentation and statistics. Data were analyzed using one-tailed Dunnett's tests. A parametric version was used if data were normally distributed as assessed by the Wilk-Shapiro test. Data which did not meet the assumptions of normality were tested using a nonparametric version of the Dunnett's test.

RESULTS

Inhibition of MLR proliferations by tepoxalin. We recently reported that tepoxalin suppresses T cell proliferation and inhibits the activity of the transcription factor NFκB (*13, 14*). T cell activation and proliferation are critical for the initiation of an antigen specific immune response. The transcription factor NFκB is also known to be involved in regulating the expression of many target genes in an immune response (*16, 17*). The possible immunosuppressive effect of tepoxalin was therefore studied. To determine whether tepoxalin is capable of inhibiting the immune response against alloantigens, tepoxalin at various concentrations was tested in MLR proliferation assays. The assay was set up by stimulating C57BL/6J (H-2^b) mouse spleen cells with irradiated B6D2F_1/J (H-$2^{b/d}$) mouse spleen cells. As shown in Figure 1, tepoxalin inhibited cell proliferation in a dose-dependent fashion with an IC_{50} of 1.3 μM. The inhibitory effect was not related to cell toxicity. Tepoxalin at concentrations of 25 μM or less did not affect the viability of anti-CD3 stimulated mouse spleen cells after 24 hr of treatment (Table 1). Since tepoxalin is a dual CO/LO inhibitor (*10*), the possible link of its suppression of MLR proliferation to its inhibition of CO and/or LO was studied. To address this question, the well-known CO inhibitor naproxen and the LO inhibitor zileuton were tested in parallel at doses 10-fold higher than their IC_{50} for suppression of CO or LO in mice, respectively. Neither of these compounds, nor the combination of both of them, had an inhibitory effect on MLR proliferation (Fig. 1).

To further understand the mechanism of action of tepoxalin, the kinetics of tepoxalin in inhibiting MLR proliferation was compared to that of the known immunosuppressant, CsA. As shown in Table 2, the inhibitory effect was not diminished when tepoxalin was added 24–72 hr after the initiation of MLR. In contrast, CsA was effective only if it was added at the beginning of the cocultures. To determine whether tepoxalin and CsA were synergistic in inhibiting MLR proliferation, the two agents were tested in combination. Tepoxalin at 0.5 μM, 1 μM, or 2 μM was tested in combination with varying concentrations of CsA (Fig. 2). CsA alone inhibited the response in a dose-related manner with an IC_{50} of 22 nM. Tepoxalin alone inhibited proliferation by 26% at 0.5 μM, by 55% at 1 μM, and by 87% at 2 μM. When tepoxalin and CsA were present at suboptimal concentrations, the inhibition was clearly additive. This additive effect was less significant at concentrations of the two drugs that were strongly inhibitory on their own.

Suppression of GVH responses by tepoxalin. The immunosuppressive effect of tepoxalin as demonstrated in MLR as-

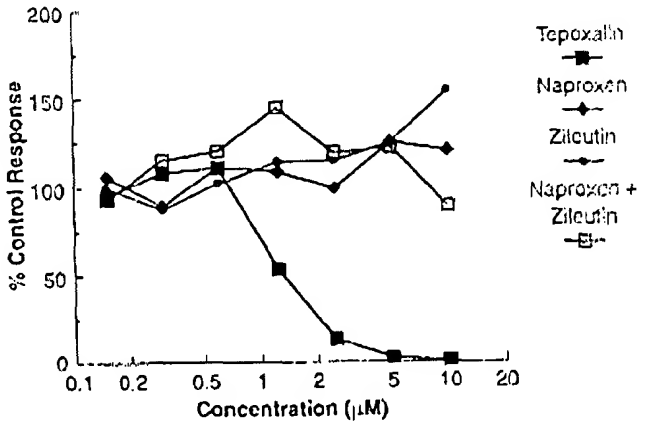

FIGURE 1. Inhibition of MLR proliferations by tepoxalin. Spleen cells from C57BL/6J mice were cocultured in triplicate wells with irradiated spleen cells from B6D2F_1/J mice as described in *Materials and Methods*. Varying concentrations of tepoxalin, naproxen, zileuton, or naproxen + zileuton were added to the cultures at the initiation of cultures. ^{3}H-thymidine uptake was measured on day 5. Control cultures contained DMSO diluted in a manner similar to that of the compounds. Uptake of ^{3}H-thymidine in vehicle controls was about 90,000 cpm. Percentages of control responses are calculated as percentages of (cpm of treated cultures/cpm of vehicle controls).

TABLE 1. The effective doses of tepoxalin in immunosuppression is not toxic to cells[a]

Tepoxalin (μM)	Cell viability[b]
100	52.0%
50	76.4%
25	96.5%
12.5	103.0%
6.25	115.5%
3.12	118.4%
1.56	136.5%

[a] Viability of anti-CD3 stimulated C57BL/GJ spleen cells treated with tepoxalin for 24 hr was tested in the MTT viability assay.

[b] Cell viability is presented as the percentage of viable cells in tepoxalin treated sample compared with that treated with an equivalent amount of the vehicle, DMSO.

TABLE 2. Inhibitory effect of tepoxalin and CsA on MLR proliferations (% control response)[a]

	Concentration (μM)	Time of Treatment			
		0 hr	24 hr	48 hr	72 hr
Tepoxalin	1.25	64.0	52.6	67.0	30.6
	2.5	17.2	12.7	19.2	15.1
	5.0	4.7	5.2	7.8	9.0
Cyclosporine	0.021	40.6	65.0	97.1	133.6
	0.042	18.6	99.6	93.2	148.0
	0.084	5.7	61.1	84.4	119.2

[a] Different concentrations of compounds added in MLR cultures at different time points were studied. MLR assays were set up as described in *Materials and Methods*. The MLR proliferations treated with compounds were compared with their vehicle controls. ^{3}H-thymidine uptake by proliferating cells in MLR assays was measured. Percentages of control responses are calculated as percentages of (cpm of treated cultures/cpm of vehicle controls).

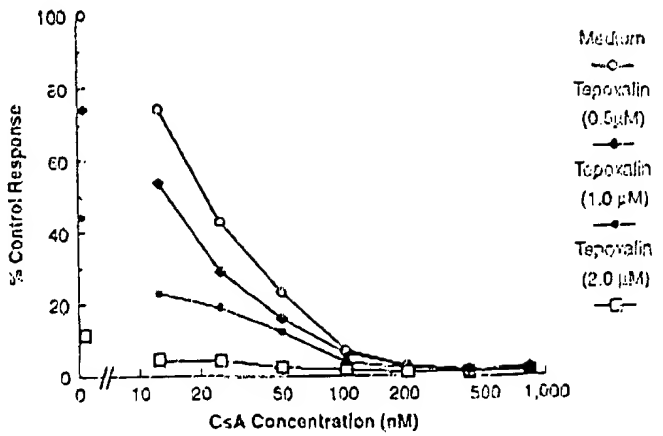

FIGURE 2. Additive inhibitory effects of tepoxalin and CsA in MLR proliferations. Proliferation of C57BL/6J mouse spleen cells after 5 days stimulation with irradiated B6D2F$_1$ spleen cells in medium containing tepoxalin at 0.5 μM, 1 μM, and 2 μM plus varying concentrations of CsA was assayed as described in *Materials and Methods*. The proliferative response in cultures containing no drugs was 80,000 cpm.

says suggests its potential use as an immunosuppressant in clinical therapy. This possible application was verified with *in vivo* murine models of transplantation. A local GVH response was performed by injecting spleen cells from the parental C57BL/GJ (H-2^b) mice into the footpads of B6D2F1/J (H-2$^{b/d}$) mice. GVH responses were demonstrated by the enlargement of the draining popliteal lymph nodes in recipient mice. The lymph nodes of recipient mice increased significantly by day 2 and continued to increase in size with time. The degree of the local GVH response was measured by weighing the draining popliteal lymph nodes. The lymph nodes of tepoxalin-treated mice did enlarge on day 2 but did not change significantly later on. After 7 days of the local GVH response, lymph nodes from tepoxalin-treated mice were slightly hyperplastic, but were significantly less so than that of the untreated controls (Fig. 3A). GVH responses in mice administered tepoxalin orally at 12–50 mg/kg/day were reduced by about 40% of that in the positive control group. Consistent with the findings in mice, tepoxalin was also effective in rats, with a 30% suppression of this local GVH response at 12 mg/kg/day (data not shown). The immunosuppressive agent CsA administered subcutaneously to mice at 50 and 75 mg/kg/day was shown to suppress GVH response by 42% and 71%, respectively (Fig. 3B). The results suggest that the immunosuppressive effect of tepoxalin at 12 mg/kg/day is comparable to that of CsA at 50 mg/kg/day. To assess whether the inhibitory effect of tepoxalin on GVH responses could be obtained with other CO or LO inhibitors, naproxen and zileuton were again tested in GVH assays. No inhibition was seen with zileuton, naproxen, or a combination of the two compounds (Fig. 3C).

Since tepoxalin appears to act late in MLR assays, the effect of tepoxalin administered early and late in GVH responses was also studied. Similar to the findings in MLR proliferations, tepoxalin given to mice for a minimum of 3 days was sufficient to suppress GVH responses to an extent similar to those treated with tepoxalin throughout the 7-day course of the GVH response (Fig. 4). This short treatment with tepoxalin could be at the early (day −1 to day 1 or 4) or the late (day 4 to day 6) stage of the GVH response. The inhibitory effect of tepoxalin at the late stage of immune responses suggests its mechanism of action to be different from that of CsA. The possible synergism in immunosuppression by tepoxalin and CsA was therefore studied in GVH assays. A much stronger suppression of the GVH response was indeed found in mice treated with both tepoxalin and CsA rather than those treated with either one of the two drugs (Fig. 5). This synergistic effect was particularly significant when a low dose of tepoxalin (6 mg/kg/day) was combined with CsA.

Prolongation of skin allograft survival by tepoxalin. The time course of skin allograft rejection in mice is affected by the efficiency of the following two mechanisms: (1) the activation of T cells through recognition of specific alloantigens, and (2) the effector mechanisms mediating tissue distruction. To study the effect of tepoxalin on skin allograft survival, experimental allograft rejection was performed by grafting allogeneic BALB/cByJ (H-2^d) mouse tail-skin onto C3H/HeJ (H-2^k) recipient mice. For the first 6 days after transplantation, allografts appeared normal and their gross appearance was not different from that of syngeneic grafts. The rejection process became apparent by day 6, with signs of swelling and erythema, and quickly culminated into complete graft necrosis. Different doses of tepoxalin were tested in skin graft rejection assays. As shown in Figure 6, rejection of allografts

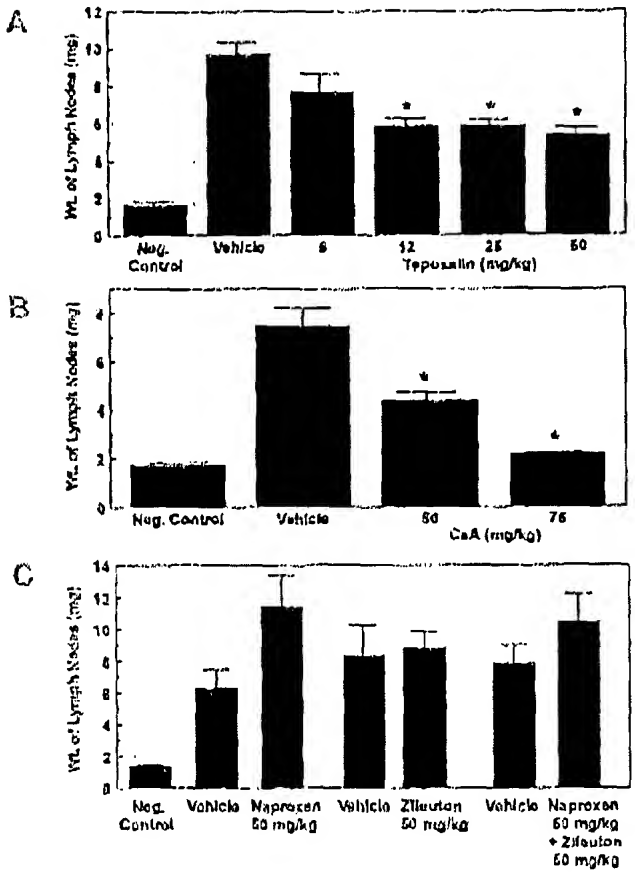

FIGURE 3. Suppression of GVH responses by tepoxalin. A local GVH response was triggered by subcutaneous injection of parental C57BL/6J spleen cells into footpads of $B6D2F_1/J$ mice, and was measured by weighing the draining lymph nodes as described in *Materials and Methods*. Mice injected with saline were used as negative controls. Drugs were given to mice from day −1 to day 6 of the GVH response. (A) GVH responses in mice administered different doses of tepoxalin or vehicle control (0.5% methylcellulose) orally. Ten mice were used per group. The values from mice treated with tepoxalin at 12, 25, and 50 mg/kg/day are significantly different from the vehicle control group (Dunnett's test). Similar results were obtained from more than three repeated experiments. (B) GVH responses in mice given CsA (50 and 75 mg/kg) or vehicle control (saline) subcutaneously. Five mice were used per group. (C) GVH responses in mice given naproxen, zileuton, or the combination of the two drugs at 50 mg/kg/day orally. Mice as vehicle controls for naproxen were treated with equivalent volumes of 0.5% methylcellulose; for zileuton, they were treated with 50% polyethylene glycol 200; and for the combination of drugs, they were treated with both 0.5% methylcellulose and 50% polyethylene glycol 200. Five mice were used per group. The column bars represent the standard errors. Asterisks indicate a P value of <0.05.

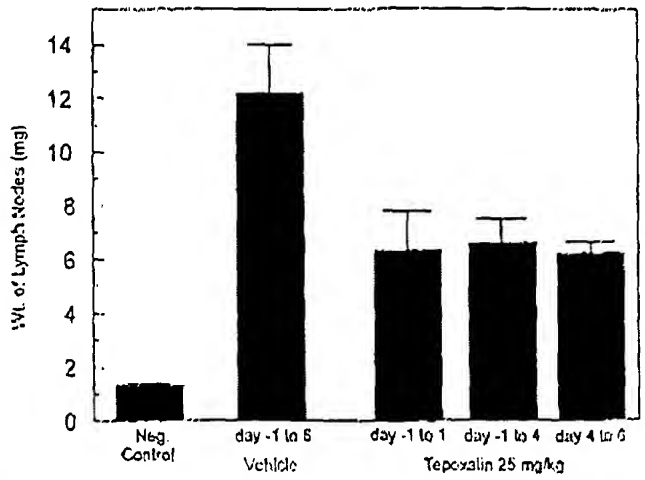

FIGURE 4. Effective suppression of mouse GVH response by short treatments with tepoxalin. The GVH response was induced by injection of C57BL/6J spleen cells into the footpads of $B6D2F_1/J$ mice and was measured by weighing the draining lymph nodes as described in *Materials and Methods*. Mice injected with saline instead of spleen cells were used as negative controls. Tepoxalin (25 mg/kg) was administered orally to mice at different time schedules as shown. GVH responses in mice treated with vehicle (0.5% methylcellulose) were used as positive controls. Five mice were used per group. The column bars represent the standard errors.

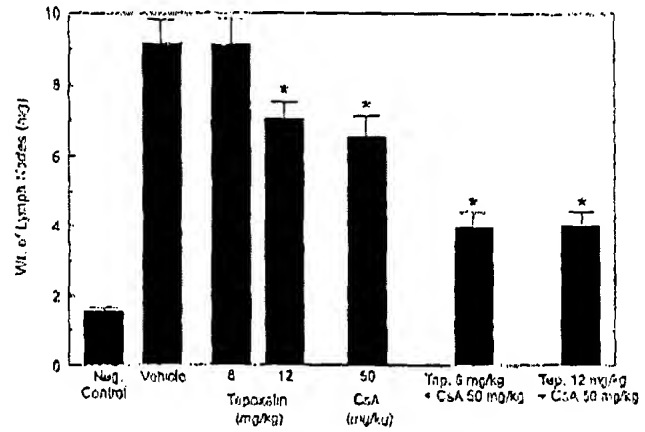

FIGURE 5. Synergistic suppression of mouse GVH responses by tepoxalin and CsA. The GVH response was induced by injection of C57BL/6J spleen cells into the footpads of $B6D2F_1/J$ mice and was measured by weighing the draining lymph nodes as described in *Materials and Methods*. Mice were treated with CsA (50 mg/kg) or tepoxalin (6 or 12 mg/kg) alone, or the combination of tepoxalin (6 or 12 mg/kg) and CsA (50 mg/kg). Mice injected with spleen cells and treated with vehicles were used as positive controls. Mice injected with saline instead of spleen cells were used as negative controls. Twenty mice were used per group. The column bars represent the standard errors. Asterisks indicate a P value of <0.05. Similar results were obtained from repeated experiments.

in the placebo-treated group started on day 7. About 50% of the allografts in the placebo group were rejected on day 10. Tepoxalin at doses of 12.5 and 25 mg/kg/day did not have a significant effect in prolonging graft rejection. When tepoxalin at 50 mg/kg/day was administered to mice, a significant prolongation of skin graft rejection was observed. The median survival time of skin grafts, defined as the time point at which 50% of the grafts are rejected, was 10.6 days in the placebo-treated group and was 15.0 days for the group of mice treated with tepoxalin at 50 mg/kg/day ($P<0.05$). Furthermore, a combination of tepoxalin and CsA at low doses showed a dramatic prolongation of allogeneic skin graft rejection (Fig. 7). About 52% of the mice treated daily with

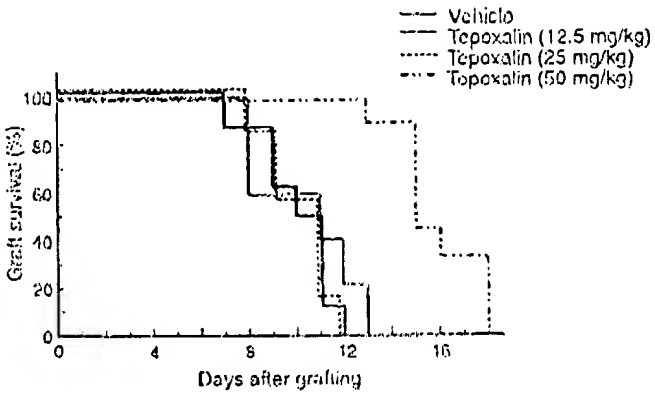

FIGURE 6. Prolongation of skin graft rejections by tepoxalin. BALB/cByJ mouse tail skin was grafted onto the tail of C3H/HeJ mice and rejection of the grafted skin was scored as described in *Materials and Methods*. Different doses of tepoxalin were administered orally to C3H/HeJ recipient mice the day before and after skin transplantation, and then daily until skin grafts were rejected. Mice given the vehicle (0.5% methylcellulose) orally were used as controls. About ten mice were used per group. Data presented were taken from one of the three repeated experiments. Results obtained from all three experiments were similar. Prolongation of skin rejection in mice treated with tepoxalin 50 mg/kg was significant ($P \leq 0.05$, Dunnett's t test).

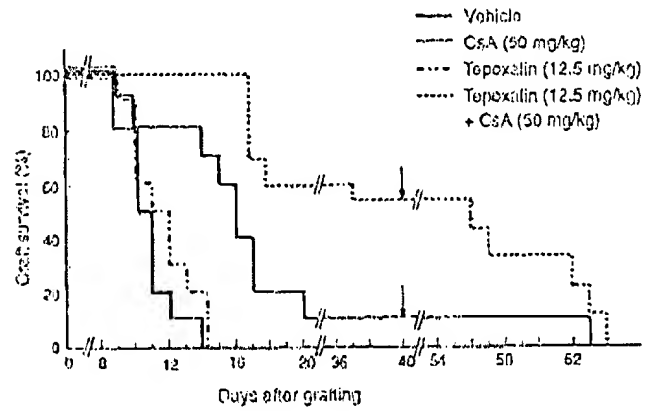

FIGURE 7. Enhanced prolongation of allogeneic skin graft rejection by tepoxalin and CsA. BALB/cByJ mouse tail skin was grafted onto the tail of C3H/HeJ mice as described in *Materials and Methods*. Tepoxalin (12.5 mg/kg) alone, CsA (50 mg/kg) alone, or tepoxalin (12.5 mg/kg) plus CsA (50 mg/kg) were administered to C3H/HeJ mice the day before and after skin transplantation, and then daily until skin grafts were rejected. Tepoxalin was given orally and CsA was given subcutaneously. For recipient mice with skin grafts surviving for more than 40 days, drug administration was discontinued from day 40, as shown by arrows. About ten mice were used per group. Enhanced prolongation of skin rejection was also observed for the combination of tepoxalin (25 mg/kg) and CsA (50 mg/kg) (data not shown).

tepoxalin (12.5 mg/kg/day) and CsA (50 mg/kg/day) retained the allogeneic skin grafts on day 40 after transplantation. To determine whether immunotolerance to skin grafts is generated by the combined drug treatment, drug dosing was discontinued after day 40 of transplantation. Skin graft rejection was noticeable on day 16 and all the grafts were rejected on day 24 after drug cessation (Fig. 7). The results suggest that the combination of tepoxalin and CsA potentiates the immunosuppressive effect, but does not induce immunotolerance to the grafts.

DISCUSSION

In this report, we demonstrate that tepoxalin is effective in suppressing the immune responses in murine models of GVH reaction and allogeneic skin graft rejection. This immunosuppressive activity is not seen with other inhibitors of CO or LO.

To study the mechanism of immunosuppression by tepoxalin, we used the *in vitro* mixed lymphocyte reaction, which measures the proliferative response of parental strain C57BL/6J spleen cells when stimulated by B6D2F$_1$/J spleen cells. Tepoxalin inhibited the alloantigen-driven proliferative response in a dose-related manner with an IC_{50} of 1.3 μM and a complete inhibition at 5 μM. A similar inhibition was seen with CsA, which had an IC_{50} of approximately 22 nM and a complete inhibition at about 200 nM. However, there were differences in the kinetics of the inhibitions seen with the two compounds. Tepoxalin exerted the same degree of inhibition if added any time up to 72 hr after the set-up of MLR cultures, CsA was only inhibitory if added at the initiation of the MLR cultures. IL-2 production by T cells occurs early following activation, reaching peak levels by 24 hr of culture (*18, 19*). CsA has been known for its inhibitory effect on IL-2 production (*7, 20, 21*) and is therefore expected to affect T cells during the first 24 hr of activation. The fact that tepoxalin inhibits proliferation late in MLR assays suggests its inhibition of later events in T cell activation. One possibility is that the IL-2-mediated signal transduction pathway is affected by tepoxalin, which has been shown on human lymphocytes in our previous report (*13*).

GVH disease is a common problem in bone marrow transplantation that leads to frequent morbidity and mortality (*22*). Skin grafts trigger strong immune responses and have been one of the most difficult grafts in transplantation (*3*). The immunosuppressive activity of tepoxalin was demonstrated in murine models of GVH responses and allogeneic skin graft rejections. Tepoxalin was found to inhibit GVH responses at 12 mg/kg/day and to prolong skin graft rejections at 50 mg/kg/day. The possibility that tepoxalin blocks a later event in immune response is again implicated by its suppression of GVH reaction even when it was administered to mice 4 days after the initiation of the response.

Tepoxalin is known to be a dual CO and LO inhibitor with potent antiinflammatory effects (*10*). One of the obvious questions to ask is whether its immunosuppression is due to the inhibition of the CO or LO enzymes. The involvement of CO and LO in the modulation of immune responses remains controversial. Arachidonic acid metabolites produced by these enzymes, such as prostaglandins and leukotrienes, have many biological activities, including the modulation of inflammation and immune response (*23–29*). Indeed several inhibitors of LO have been shown to prolong graft rejection in transplantation (*30–33*). However, it was noticed that those LO inhibitors with immunosuppression activity are also potent antioxidants with inhibitory effects on NFκB activity (*34, 35*). Therefore the immunoregulatory effects of these

compounds may not be directly related to inhibition of LO. We compared the effect of tepoxalin with other known LO/CO inhibitors in our studies. Naproxen (CO inhibitor) or zileuton (LO inhibitor), or their combination, did not have any effect on MLR proliferations or GVH responses. We have reported recently that tepoxalin is distinct from other CO and LO inhibitors in its inhibition of NFκB activities (*14*). NFκB is a pleiotropic transactivator of many target genes involved in immune or inflammatory responses (*16, 17*). The immunosuppressive effect of tepoxalin may be attributed to its inhibition of NFκB and not related to the general inhibition of arachidonic acid metabolism.

Taken together, these data show that tepoxalin is an effective immunosuppressive agent. Since the mechanism of tepoxalin appears to be different from CsA in immunosuppression, it suggests a possible combinational use of the two compounds in immunosuppressive therapy. Moreover, tepoxalin is devoid of ulcerogenic actions in gastrointestinal systems that are the common side effects of other NSAID drugs (*11, 12*). The LD_{50} of tepoxalin in mice and rats was more than 400 mg/kg, which is over 10-fold higher than the effective doses used in *in vivo* immunosuppression. Tepoxalin could therefore be an important addition to the existing immunosuppressive therapeutic drugs to enhance the efficacy of treatment and to reduce drug toxicity in transplantation and autoimmunity.

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COMBINED THERAPY WITH INTERLEUKIN-4 AND INTERLEUKIN-10 INHIBITS AUTOIMMUNE DIABETES RECURRENCE IN SYNGENEIC ISLET-TRANSPLANTED NONOBESE DIABETIC MICE

Analysis of Cytokine mRNA Expression in the Graft[1]

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Syngeneic pancreatic islet grafts in nonobese diabetic (NOD) mice elicit a cell-mediated autoimmune response that destroys the insulin-producing β cells in the islet graft. IL-4 and IL-10 are cytokines that inhibit cell-mediated immunity. In this study, we evaluated the effects of IL-4 and IL-10 on the survival of syngeneic pancreatic islets transplanted into diabetic NOD mice. Islet grafts survived beyond 18 days and normoglycemia was maintained in 67% (10 of 15) of mice treated with IL-4 plus IL-10, but in none (0 of 20) of vehicle-injected (control) mice. Also, 40% (6 of 15) of the mice treated with IL-4 plus IL-10 were normoglycemic at 80 days after transplantation, compared with 14% (1 of 7) of the mice treated with IL-4 alone, 8% (1 of 13) of the mice treated with IL-10 alone, and none (0 of 20) of the control mice. Histological examination of grafts at 10 days after transplantation revealed perinislet accumulations of mononuclear leukocytes and intact islet β cells in grafts from IL-4 plus IL-10-treated mice, whereas islets were infiltrated by leukocytes and the β cell mass was greatly reduced in grafts from control mice. Polymerase chain reaction (PCR) analysis of cytokine mRNA expression in the grafts revealed higher levels of IL-2, IFNγ, and IL-10 mRNA in grafts of diabetic compared with normoglycemic control mice, whereas IFNγ and TNFα mRNA levels were significantly decreased in grafts of IL-4 plus IL-10-treated mice compared with either normoglycemic or diabetic control mice. These results suggest that T helper (Th)1 cells and their cytokine products (IL-2, IFNγ, and TNFα) may promote islet β cell destructive insulitis and autoimmune diabetes recurrence in syngeneic islet-transplanted NOD mice, and that administration of IL-4 plus IL-10 may inhibit diabetes recurrence by suppressing Th1 cytokine production in the islet grafts.

Insulin-dependent diabetes mellitus (IDDM)* results from destruction of the insulin-producing pancreatic islet β cells by the host's own immune system. Whereas it is not known what may initiate this autoimmune response against islet β cells, there is abundant evidence that IDDM is T cell-dependent (*1, 2*). However, it is unclear which T cells are involved and how they may lead to islet β cell destruction. A variety of immune/inflammatory cells infiltrate the pancreatic islets and constitute the insulitis lesion (*3, 4*). There is evidence in human patients with IDDM (*5–8*) and in animals with spontaneous IDDM resembling the human disease—the nonobese diabetic (NOD) mouse and the biobreeding (BB) rat (*9–22*)—that islet β cell destruction may involve heterogeneous effec-

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* Abbreviations: BB, Biobreeding; CFA, complete Freund's adjuvant; IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; PCR, polymerase chain reaction; Th, T helper.

1: J Immunol Methods. 1994 Feb 10;168(2):149-65. Related Articles, Links

The genetic engineering of monoclonal antibodies.

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A number of recent technological developments have greatly facilitated the genetic engineering of immunoglobulins. The use of PCR has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunised cells. The conversion of the rodent antibody into a humanized version is now well established. To develop these antibodies for clinical use has required the development of high level expression systems. For the expression of large multimeric glycoproteins, mammalian cell systems generally provide the highest levels of secreted product and therefore are the methods of choice for producing whole recombinant antibodies. Novel antigen-binding units have been developed by joining the two variable domains of an antibody into single-chain polypeptides. Such fragments can be produced in high yield by secretion from E. coli raising the prospect of bulk preparation of these antibody fragments for the development of low-cost immunopurification and assay reagents. Finally, the ability to screen for antigen binding by displaying immunoglobulin variable regions on the surface of filamentous bacteriaphages has opened up the possibility of bypassing the immune system to generate novel antibody specificities in vitro.

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SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

Contents

SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

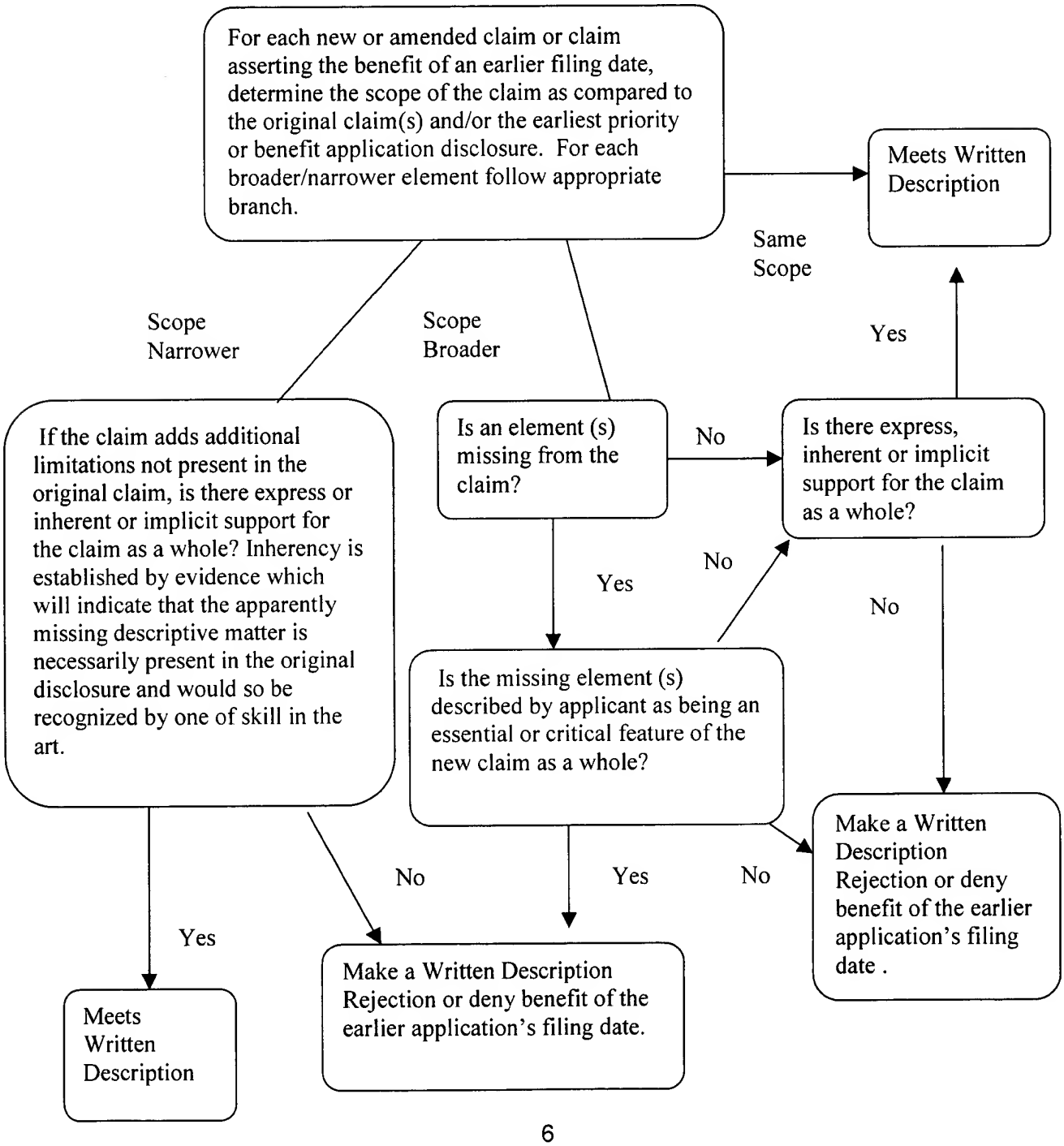
It is assumed at this point in the analysis that the specification has been reviewed and an appropriate search of the claimed subject matter has been conducted. It is also assumed that the examiner has identified which features of the claimed invention are conventional taking into account the body of existing prior art. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. If the examiner determines that the application does not comply with the written description requirement, the examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. It should also be noted that the test for an adequate written description is separate and distinct from the test under the enablement criteria of 35 U.S.C. § 112 first paragraph. The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

The following examples only describe how to determine whether the written description requirement of 35 U.S.C. 112, para. 1 is satisfied. Regardless of

the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of Title 35 of the U.S. Code. Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

Written Description Amended or New Claims, or Claims Asserting the Benefit of an Earlier Filing Date

Decision Tree



Written Description

Original Claims

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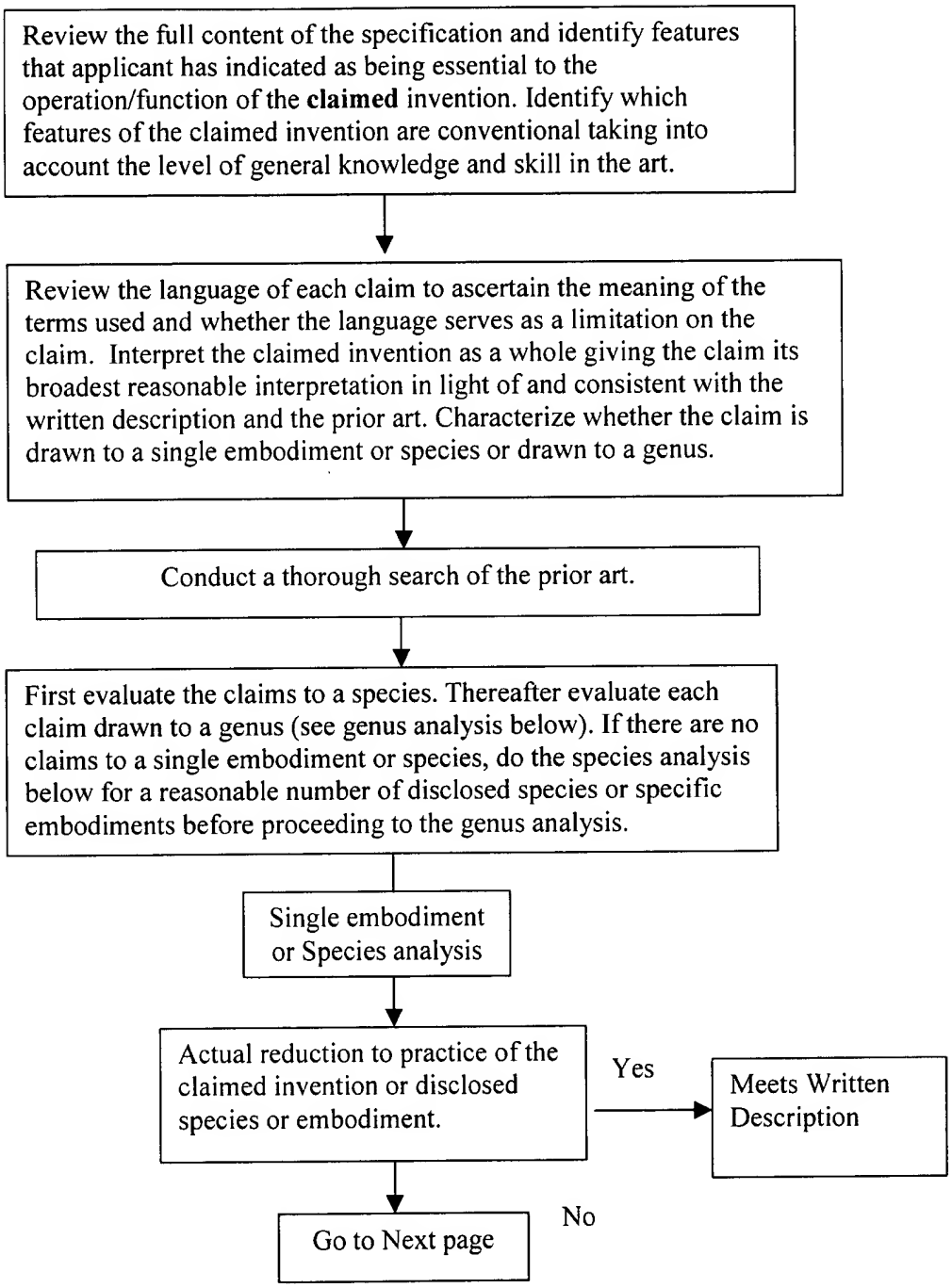

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Original Claims

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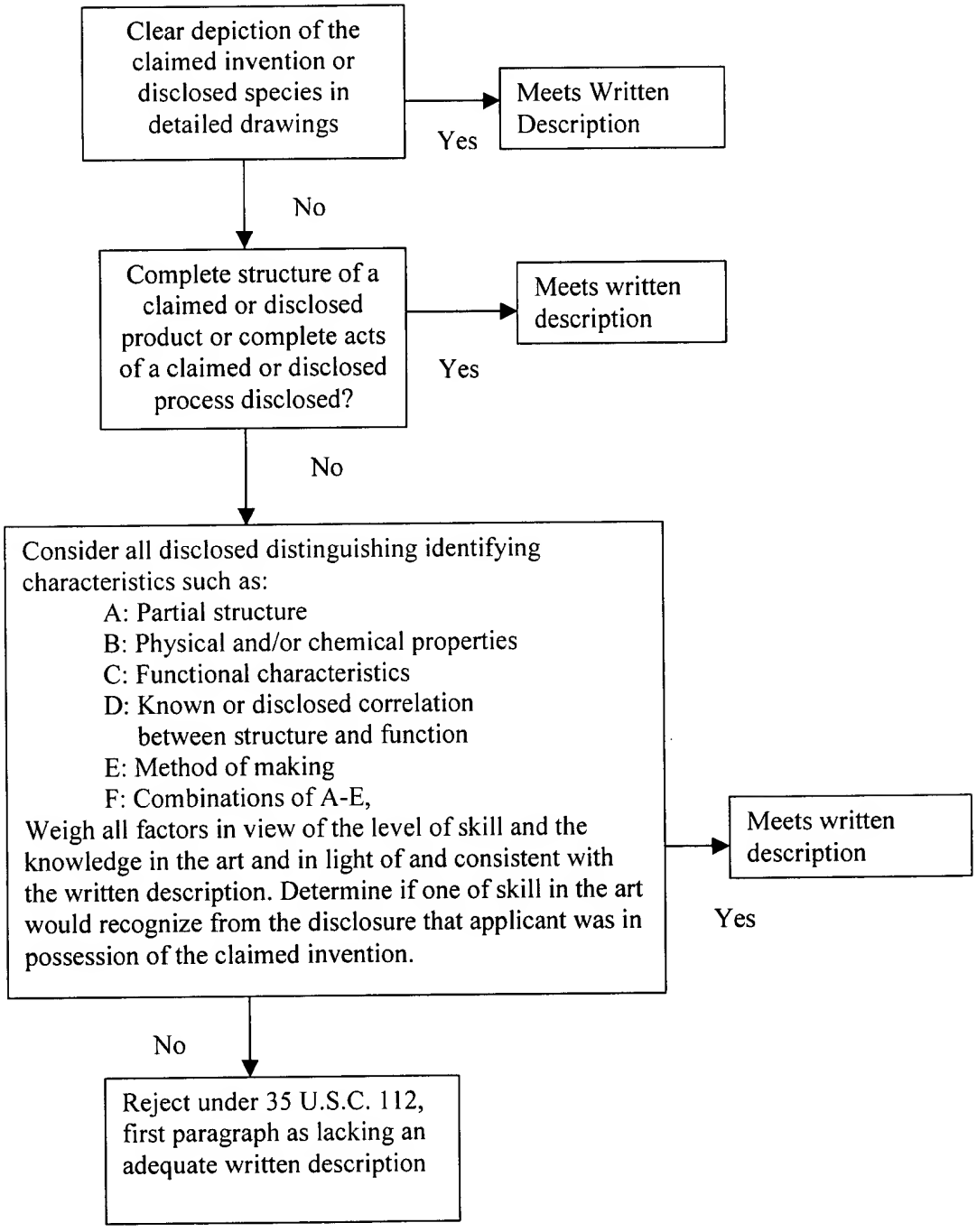
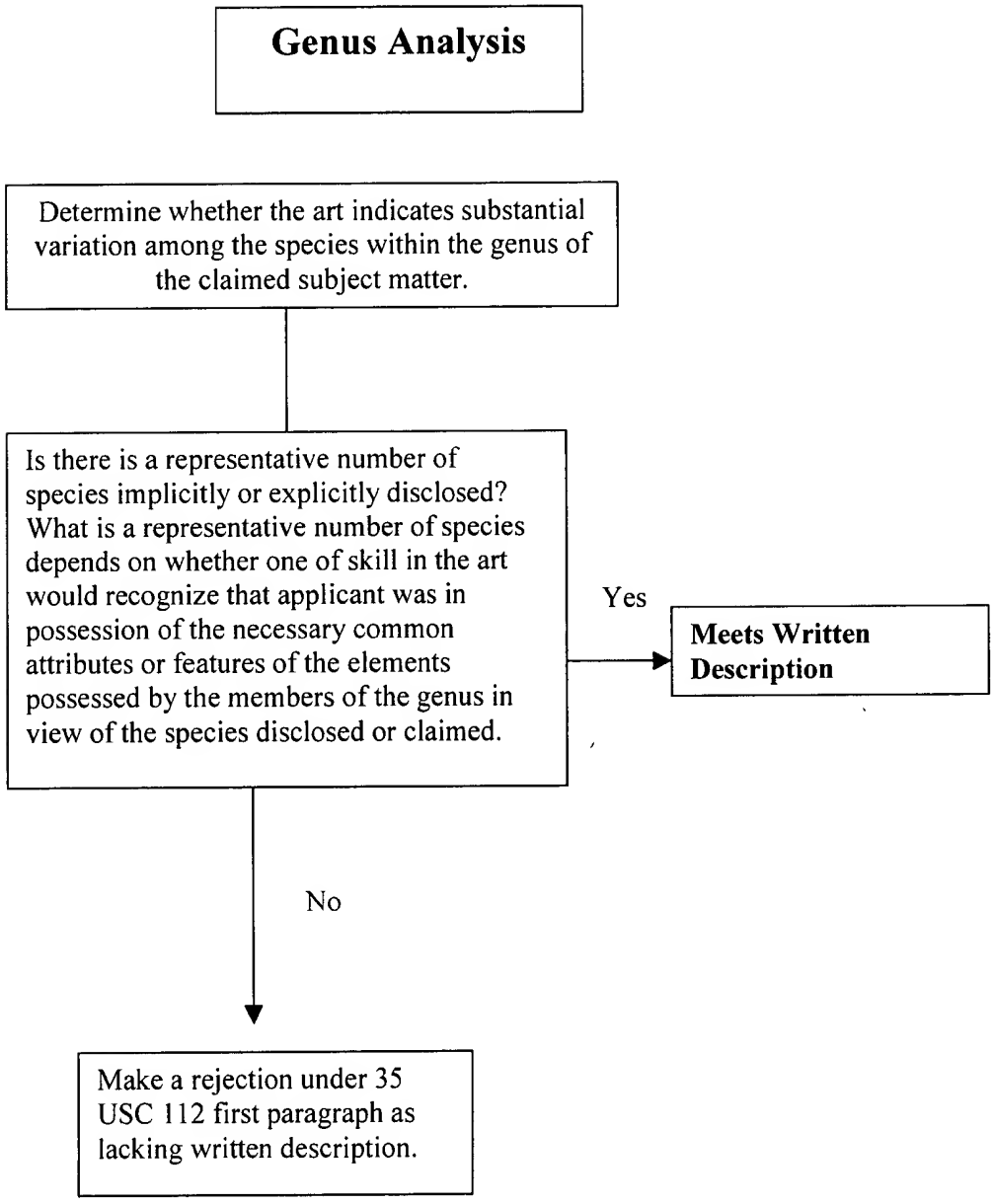


Written Description

Original Claims

Decision Tree

--Page 3--



WRITTEN DESCRIPTION TRAINING EXAMPLES

Example 1: Amended claims

Fact Pattern:

The specification is directed to a sectional sofa with a console between two reclining chairs, wherein control means for the reclining chairs are mounted on the console. The original disclosure clearly identifies the console as the only possible location for the controls, and provides for only the most minor variation in the location of the controls, e.g., the controls may be mounted on the top or side surfaces of the console or on the front wall. Additionally, the specification states that the purpose for the console is to house the controls. The original claims required the control elements to be present in the console. Applicant subsequently amends the claims to remove this limitation.

Amended Claim:

1. (Amended) A sectional sofa comprising:

a pair of reclining seats disposed in parallel relationship with one another in a double reclining seat sofa section, said double reclining seat sofa section being without an arm at one end whereby a second sofa section of the sectional sofa can be placed in abutting relationship with the end of the double reclining seat sofa section without an arm so as to form a continuation thereof,

each of said reclining seats having a backrest and seat cushion and movable between upright and reclined positions, said backrests and seat cushions of the pair of reclining sets lying in respective common planes when the seats are in the same positions,

a fixed console disposed in the double reclining seat sofa section between the pair of reclining seats and with the console and reclining seats together comprising a unitary structure, said console including an armrest portion for each of the reclining seats, said arm rests remaining fixed when the reclining seats move from one to another of their positions, and

a pair of control means [located upon the center console to enable each of the pair of reclining seats to move separately between the reclined and upright positions] mounted on the double reclining seat sofa section and each readily accessible to an occupant of its respective reclining seat and when actuated causing the respective reclining seat to move from the upright to the reclined position.

Analysis:

The amended claim is broader than the original claim in that the pair of control means is no longer required to be located on the center console. Thus, control means mounted on a center console is an element missing from the claim. The specification describes the location of the control means on the console as an essential feature of the claimed invention as a whole because the specification clearly identifies the console as the only possible location for the controls, and states that the purpose for the console is to house the controls.

Conclusion:

Reject the amended claim under 35 USC §112 first paragraph as lacking adequate written description.

Example 2: 35 USC 120 Priority

Fact Pattern:

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup is not important, as long as the implant can effectively function as an artificial hip socket. The application is a continuation in part of a parent application that describes an acetabular cup prosthesis wherein the cup is a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The parent specification also touts the criticality of a conical cup over all other shape cups.

A reference disclosing the claimed invention published between the filing date of the parent application and the instant application. Applicant asserts entitlement to the filing date of the parent application.

Claim:

1. An acetabular cup prosthesis comprising (1) a body extending generally longitudinally and terminating into front and rear surfaces, said front surface extending substantially transversely to said body; and (2) at least one fin for securing said cup to a prepared acetabulum cavity, said fin having a length extending generally longitudinally from said front surface toward said rear surface continuously along said body throughout the entire length of said fin, and said fin being configured so as to extend radially outwardly beyond the perimeter of said front surface and said body so as to engage with the cavity thereby securing said cup.

2. The prosthesis of claim 1, wherein the body has a generally conical outer surface.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the parent application, which only describes a conical cup. Claim 1 is missing the element of a conical shape. This element is an essential or critical feature of the invention described in the parent application because the parent application only discloses a conical shape and the conical shape is described as critical over other shapes.

Claim 2 of the instant application is directed to an acetabular cup prosthesis wherein the cup has a generally conical outer surface. The claim is of the same scope as the invention described in the parent application.

Conclusion:

Reject claim 1 over the prior art reference, and indicate that the claim is not entitled to the benefit of the earlier application filing date.

Indicate that claim 2 is entitled to the benefit of the parent application filing date.

Note that if applicant had added the subject matter of claim 1 of this application to the parent application in an amendment, the claim would have been rejected under 35 U.S.C. 112, first paragraph as lacking an adequate written description.

Example 2A: Essential element missing from original claim

Fact Pattern:

The fact situation of example 2 above is similar to the fact situation of the instant example, however, there is no parent application in this example.

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup is critical to permit the implant to effectively function as an artificial hip socket. The application describes an acetabular cup prosthesis wherein the cup is a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The specification also touts the criticality of a conical cup.

Claims: Same as claims 1 and 2 of example 2 above.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the instant application that only describes a conical cup. Claim 1 is missing the element of a conical shape. A review of the specification indicates that a cup implant having a shape which can effectively function as an artifical hip socket is critical to the operation/function of the claimed invention. The application discloses a conical shape cup and the conical shape is described as critical over other shapes. The specification indicates that the invention **as claimed** will not function in its intended manner without the specific cup

shape. Therefore this element is essential to the function/operation of the invention.

Claim 1 is directed to a genus. There is no actual reduction to practice or clear depiction of the claimed invention in detailed drawings; however, the complete structure of a species of the claimed prosthesis (with conical shape) is disclosed. The disclosed species is not representative of the genus because the specification indicates that without the conical shape the invention will not operate as intended. Therefore, applicant was not in possession of the necessary common attributes of the elements possessed by the members of the genus. A written description rejection should be made in this situation.

Example 2B: A preferred element missing from original claim

Fact Pattern:

The fact situation of example 2B is similar to example 2A above except that in this example the shape of the conical cup is described as being preferred.

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup must permit the implant to effectively function as an artificial hip socket. The application describes an acetabular cup prosthesis wherein the cup is preferably a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The specification emphasizes that a conical cup is the preferred embodiment.

Claims: Same as claims 1 and 2 of example 2 above.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the instant application that only describes a conical cup. Claim 1 is missing the element of a conical shape. A review of the specification indicates that a cup implant having a conical shape is preferred but has no apparent bearing to the operation/function of the claimed invention. Therefore this element is not essential to the function or operation of the invention.

Claim 1 is directed to a genus. Although there is no actual reduction to practice or clear depiction of the claimed invention in detailed drawings, the complete structure of a species of the claimed prosthesis (with conical shape) is disclosed. The disclosed species is representative of the genus because there is a known correlation between the structure and the function of claimed invention and one of skill in the art would recognize that applicant was in possession of the necessary common attributes of the elements possessed by the members of the genus. The invention as claimed will function in its intended manner even without the specific cup shape. No written description rejection should be made in this situation.

Note: ***If the specification needs to be amended to be consistent with an original claim, see MPEP 608.01(o).***

Example 3: <u>New claims</u>

Fact Pattern:

The specification describes a form of computer technology called multi-threading. In essence, computers with multi-threading capabilities can switch between tasks with such rapidity that they appear to be performing two or more tasks at once. The specification describes one illustrative example in the specification wherein one of the program threads is an editor and another thread is a code processing routine in the form of a compiler. As the operator strikes keys at the keyboard, the compiler thread executes between each successive pair of keystrokes to process the entered source code concurrently with the editing operation. By the time the operator has finished entering or editing the code the compiler thread will have completed most of the required processing, thereby freeing the operator from lengthy periods of waiting for extensive code processing.

In this illustrative embodiment the interrupt operation of the central processor is periodically activated by a timer or clock. Each interrupt operation asynchronously preempts the executing compiler thread and passes control of the central processor to an interrupt service routine. The input port is then polled to test if a key has been struck at the keyboard. If not, the interrupt is terminated and control returns to the compiler thread. If polling the port reveals that a key has been struck then the interrupt service routine invokes the editor thread which takes control of the central processor to perform a character code entry or other edit operation. In addition to the description above, the application's abstract references an editor, compiler, interrupt means, and return means, and the "Object of the Invention" section

and the "Description of Prior Art" clearly discuss the importance of an editor and compiler.

The original claims required, *inter alia*, an editor, a compiler, an interrupt means and a return means. These elements are missing from new claim 20.

Claim:

20. A computer-readable disk memory having a surface formed with a plurality of binary patterns constituting a multithreaded application program executable by a desktop computer having a central microprocessor, a memory, means for loading said application program into a defined address space of said memory, and a clock-driven periodically-activated interrupt operation, said multithreaded program comprising

a plurality of sets of instructions with each set executable by said microprocessor,

a first of said sets of instructions executable to provide a first thread of execution having control of the central microprocessor,

said first thread of execution being periodically preempted in response to activations of an interrupt operation at predetermined fixed time intervals, and

a second of said sets of instructions executable to provide a second thread of execution to acquire control of the central microprocessor,

each of said threads having direct access to said program memory address space so as to provide fast efficient preemption of one thread by

another thread and switching of control of the central microprocessor back and forth among the threads at a rate so rapid that the threads execute effectively simultaneously.

Analysis:

Claim 20 is a new claim, which is broader in scope than the original claims. There are four elements missing from the claims (the editor, compiler, interrupt means, and return means). These missing elements are described by applicant as being an essential or critical feature of the claimed invention as a whole as evidenced by applicant's repeated reliance on the presence of these elements throughout the originally filed disclosure. Multiple sections within the application make clear that these four elements served integral functions in the overall invention.

Conclusion:

Reject claim 20 as lacking an adequate written description because four elements described as essential or critical are omitted. The omitted elements are: editor, compiler, interrupt means, and return means.

Example 4 : **Original claim**

Fact Pattern:

The invention is directed to a form of autopilot, described as a "heading lock," which enables a person to maintain directional control over a watercraft without constant manipulation of trolling motor controls. The preferred embodiment, as set forth in the written description and clearly depicted in detailed drawings, employs a compass mounted to the head of the "heading lock" unit, which monitors the direction of the thrust motor. The heading lock is coupled to the trolling motor; in a preferred embodiment, the heading lock is mechanically coupled to the trolling motor. The disclosure specifically notes that the direction of the thrust motor is considered to be the same as the direction of the boat since the trolling motor is mounted on the bow of the boat. The specification indicates that the electronic steering system continues to monitor the current heading of the thrust and also indicates that the heading detector continuously monitors the current heading of the boat. The term "heading" is used interchangeably throughout the written description to refer to both the direction of the trolling motor and the direction of the boat.

Claim:

1. A heading lock coupled to a trolling motor producing a thrust disposed to pull a watercraft, said heading lock comprising:

a steering motor coupled to said trolling motor, said steering motor being disposed to affect the orientation of said trolling motor in response to input signals;

a steering circuit electrically coupled to said steering motor, said steering circuit being disposed to generate said input signals to said steering motor in response to heading signals; and

a heading detector electrically coupled to said steering circuit, said heading detector being disposed to transmit said heading signals to said steering circuit.

Analysis:

Applicant has identified a heading lock comprising a steering system coupled to a trolling motor and a heading detector, as features essential to the operation of the claimed invention. Although the heading lock is preferably mechanically coupled to the trolling motor, the applicant does not describe the type of coupling as essential to the claimed invention as a whole. A search of the prior art shows that various means for coupling a heading lock to a trolling motor are conventional in the art. The claim is drawn to a single embodiment. Although there is no reduction to practice of the claimed invention, the claimed invention is clearly depicted in detailed drawings.

Conclusion:

The claim is adequately described.

Example 5: Flow Diagrams

Fact Pattern:

The specification is directed to a mechanism for controlling the mode of operation of a modem. A modem is used for modulating and demodulating signals, both analog and digital, over telephone lines. It has two modes: (1) a transparent mode, in which the modem performs the modulation-demodulation function, and (2) a command mode, in which the modem responds to predetermined commands and performs operations by executing a set of instructions stored in Read-Only-Memory (ROM) or firmware. An escape command tells the modem when to switch between transparent and command modes.

The application claims an improved mechanism for detecting an escape command by a modem. The decision making capability and timing means preferably reside in a microprocessor, preferably a Z-8 type microprocessor. The specification discloses logic flow diagrams and provides a detailed functional recitation that describes how to program computers to detect an escape command, but the specification does not provide a computer program listing with source code. The specification describes the escape sequence as one full second of no data, followed by the predetermined escape command, followed by another full second of no data.

Claim:

1. In a modem including a data input port for connecting said modem to a utilization device, and a telephone port for connecting said modem to a

telephone line, said modem being of the type having two distinct modes of operation:

(a) a transparent mode of operation for which said modem provides modulated signals to said telephone port in response to data signals provided to said data input port; and

(b) a command mode of operation for which said modem responds to said data signals provided to said data input port as instructions to said modem;

said modem including means defining a predetermined sequence of said data signals as an escape character; the improvement comprising:

timing means for detecting each occurrence of a passage of a predetermined period of time after provision of one of said data signals to said data input port; and

means, operative when said modem is in said transparent mode of operation, for detecting provision of said predetermined sequence of said data signals, and for causing said modem to switch to said command mode of operation, if and only if said predetermined sequence of data signals occurs contiguous in time with at least one said occurrence of said passage of said predetermined period of time during which none of said data signals are provided to said data input port.

Analysis:

After a review of the full content of the specification, the examiner finds that a modem having two modes of operation (transparent and

command), a timing means, and a means for detecting an escape sequence and causing the modem to switch from the transparent to the command mode are essential to the operation and function of the claimed invention. The specification does not describe a particular timing means or means for detecting the escape command and switching to the command mode. The claim is drawn to a genus. A search of the prior art indicates that the structure of the hardware required is conventional, and that one skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. A review of the art indicates that there is no substantial variation among the species within the genus. Although no embodiments have been actually reduced to practice, a review of the specification shows that the claimed invention has been reduced to drawings in view of the detailed functional flow diagrams. Since the claimed invention is supported by conventional hardware structure and because there is a functional description of what the software does to operate the computer, there is sufficient description of the claimed invention. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.

Conclusion:

The claimed invention has been adequately described.

Biotechnology Examples

Example 6: Genes

Specification: The specification describes an isolated cDNA fragment (SEQ ID NO: 1; a 100mer) obtained from a human glioblastoma cDNA library. SEQ ID NO: 1 is asserted to be homologous to a known DNA molecule that encodes the extracellular domain of a glial specific G-coupled protein receptor whose function is associated with glial cell differentiation. The observed homology is sufficient to support a conclusion that SEQ ID NO: 1 would be glial specific. Further, it would be reasonable to infer that a G-coupled protein receptor encoded by a cDNA that comprised SEQ ID NO: 1 would be involved in the regulation of glial cell differentiation. In the description, applicant defines a "gene" as including naturally occurring regulatory elements and untranslated regions necessary and sufficient to mediate the expression of a cDNA comprising SEQ ID NO: 1. The specification describes methods for cloning nucleic acids that encode full-length glial specific G coupled protein receptors. The specification also discloses that SEQ ID NO: 1 can be used as a probe for identifying the presence of nucleic acids encoding glial specific G-coupled protein receptors in mammals. Glial specific G-coupled protein receptors are disclosed as useful in drug discovery methods to identify agents that regulate glial differentiation. The specification defines a probe as consisting of SEQ ID NO: 1 and between five to 10 additional nucleotides on either end of SEQ ID NO: 1.

Claim:

An isolated gene comprising SEQ ID NO: 1.

Analysis:

A review of the specification indicates that elements which are not particularly described, including regulatory elements and untranslated regions, are essential to the function of the claimed invention because applicant's definition of "gene" requires them. Additionally, SEQ ID NO: 1 is disclosed as being essential to the function of the claimed invention. The art indicates that the structure of genes with naturally occurring regulatory elements and untranslated regions is empirically determined. For example, the structural elements of "gene" mediating the expression of a particular protein in the liver may be different than the structural elements of the "gene" mediating the expression of the same protein in the brain. Therefore the structure of these elements which applicant considers as being essential to the function of the claim are not conventional in the art.

The claim is drawn to a genus, i.e., any gene which comprises SEQ ID NO: 1.

A search of the prior art indicates that SEQ ID NO: 1 is otherwise novel and unobvious, and no associated genomic clones have been identified.

There is no actual reduction to practice of the claimed invention, clear depiction of the claimed invention in the drawings or complete detailed description of the structure.

Considering all disclosed distinguishing identifying characteristics, there is a disclosure of partial structure (SEQ ID NO: 1) as well as the function of the gene as coding for a G-coupled protein receptor.

However, there is no known or disclosed correlation between this function and the structure of the non-described regulatory elements and untranslated regions of the gene. Furthermore, there is no additional disclosure of physical and/or chemical properties. Weighing all factors in view of the level of knowledge and skill in the art, one skilled in the art would not recognize from the disclosure that the applicant was in possession of the genus of genes which comprise SEQ ID NO: 1.

Conclusion:

Reject claim 1 under 35 USC 112 first paragraph as lacking an adequate written description. The examiner should make a rejection following a similar type of reasoning as that set forth above.

Note: Applicant may overcome this rejection by claiming a probe which consists essentially of SEQ ID NO: 1, since the specification teaches that a probe can have no more than 10 additional nucleic acid residues at either end of the molecule. The examiner should make an express determination that "consisting essentially of" admits of no more than 10 additional residues at either end of the molecule.

Example 7: EST

Specification: The specification discloses SEQ ID NO: 16 which is a partial cDNA. The specification does not address whether the cDNA crosses an exon/intron splice junction. The specification discloses that this sequence will specifically hybridize with the complement of the coding sequence of a gene of an infectious yeast. The presence of the nucleic acid detected by hybridization with the complement of the coding sequence is useful for identifying yeast infections. Example 1 of the specification describes an experiment where SEQ ID NO: 16 was determined following characterization of a cDNA clone isolated from a cDNA library.

Claim:

An isolated DNA comprising SEQ ID NO: 16.

Analysis:

A review of the full content of the specification indicates SEQ ID NO: 16 is essential to the operation and function of the claimed invention. The specification indicates that the presence of DNA that hybridizes with SEQ ID NO: 16 is indicative of a yeast infection.

A review of the language of the claim indicates that the claim is drawn to a genus, i.e., any nucleic acid that minimally contains SEQ ID NO: 16 within it including any full length gene which contains the sequence, any fusion constructs or cDNAs.

The search indicates that SEQ ID NO: 16 is a novel and unobvious sequence.

There is a single species explicitly disclosed (a molecule consisting of SEQ ID NO: 16 that is within the scope of the claimed genus).

There is actual reduction to practice of the disclosed species.

The disclosure of a single disclosed species may provide an adequate written description of a genus when the species disclosed is representative of the genus. The present claim encompasses full-length genes and cDNAs that are not further described. There is substantial variability among the species of DNAs encompassed within the scope of the claims because SEQ ID NO: 16 is only a fragment of any full-length gene or cDNA species. When reviewing a claim that encompasses a widely varying genus, the examiner must evaluate any necessary common attributes or features. In the case of a partial cDNA sequence that is claimed with open language (comprising), the genus of, e.g., "A cDNA comprising [a partial sequence]," encompasses a variety of subgenera with widely varying attributes. For example, a cDNA's principle attribute would include its coding region. A partial cDNA that did not include a disclosure of any open reading frame (ORF) of which it would be a part, would not be representative of the genus of cDNAs because no information regarding the coding capacity of any cDNA molecule would be disclosed. Further, defining "the" cDNA in functional terms would not suffice in the absence of a disclosure of structural features or elements of a cDNA that would encode a protein having a stated function.

A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a

substantial portion of the genus. Regents of the University of California v. Eli Lilly & Co., 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Here, the specification discloses only a single common structural feature shared by members of the claimed genus, i.e., SEQ ID NO: 16. Since the claimed genus encompasses genes yet to be discovered, DNA constructs that encode fusion proteins, etc., the disclosed structural feature does not "constitute a substantial portion" of the claimed genus. Therefore, the disclosure of SEQ ID NO: 16 does not provide an adequate description of the claimed genus.

Weighing all factors, 1) partial structure of the DNAs that comprise SEQ ID NO: 16, 2) the breadth of the claim as reading on genes yet to be discovered in addition to numerous fusion constructs and cDNAs, 3) the lack of correlation between the structure and the function of the genes and/or fusion constructs; in view of the level of knowledge and skill in the art, one skilled in the art would not recognize from the disclosure that the applicant was in possession of the genus of DNAs which comprise SEQ ID NO: 16.

Conclusion: The written description requirement is not satisfied.

Caveat: *In situations where the specification indicates that the SEQ ID NO: is a full-length cDNA open reading frame and the claim* ***cannot*** *read on a gene, the claimed invention would meet the written description requirement.*

Example 8: <u>DNA fragment Encoding a Full Open Reading Frame (ORF)</u>

Specification: The specification discloses that a cDNA library was prepared from human kidney epithelial cells and 5000 members of this library were sequenced and open reading frames were identified. The specification discloses a Table that indicates that one member of the library having SEQ ID NO: 2 has a high level of homology to a DNA ligase. The specification teaches that this complete ORF (SEQ ID NO: 2) encodes SEQ ID NO: 3. An alignment of SEQ ID NO: 3 with known amino acid sequences of DNA ligases indicates that there is a high level of sequence conservation between the various known ligases. The overall level of sequence similarity between SEQ ID NO: 3 and the consensus sequence of the known DNA ligases that are presented in the specification reveals a similarity score of 95%. A search of the prior art confirms that SEQ ID NO: 2 has high homology to DNA ligase encoding nucleic acids and that the next highest level of homology is to alpha-actin. However, the latter homology is only 50%. Based on the sequence homologies, the specification asserts that SEQ ID NO: 2 encodes a ligase.

Claim 1: An isolated and purified nucleic acid comprising SEQ ID NO: 2.

Analysis:

A review of the full content of the specification indicates SEQ ID NO: 2 is essential to the operation and function of the claimed invention. The specification indicates that SEQ ID NO: 2 encodes a protein that would be expected to act as a DNA ligase.

A review of the language of the claim indicates that the claim is drawn to a genus, i.e., any nucleic acid that minimally contains SEQ ID NO: 2. The claim is drawn to a nucleic acid comprising a full open reading frame. The claimed nucleic acid does not read on a genomic sequence because full-length mammalian cDNAs would not be expected to contain introns or transcriptional regulatory elements such as promoters that are found in genomic DNA. The claim reads on the claimed ORF in any construct or with additional nucleic acid residues placed at either end of the ORF.

The search indicates that SEQ ID NO: 2 is a novel and unobvious sequence.

There is a single species explicitly disclosed (a molecule consisting of SEQ ID NO: 2 that is within the scope of the claimed genus).

There is actual reduction to practice of the disclosed species.

One of skill in the art can readily envisage nucleic acid sequences which include SEQ ID NO: 2 because e.g. SEQ ID NO: 2 can be readily embedded in known vectors. Although there may be substantial variability among the species of DNAs encompassed within the scope of the claim because SEQ ID NO: 2 may be combined with sequences known in the art,

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: Hybridization

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

Example 10: Process claim

Specification: The specification teaches that SEQ ID NO: 10 is an EST. The specification also teaches that SEQ ID NO: 10 is a chromosome marker and that any DNA which hybridizes under specified stringent conditions to SEQ ID NO: 10 will be useful as a marker for detecting the presence of Burkitt's lymphoma. The specification also teaches how to produce DNAs including genomic DNAs which hybridize to SEQ ID NO: 10 and isolation of said DNAs. The specification presents an example where a genomic DNA is probed with SEQ ID NO: 10 under the specified stringent conditions (6XSSC and 65 degrees Celsius) and the genomic DNA which hybridizes under these conditions is isolated and is sequenced. The sequence of this genomic clone is represented by SEQ ID NO: 11.

Claim:

Claim 1: A process for producing an isolated polynucleotide comprising hybridizing SEQ ID NO: 10 to genomic DNA in 6XSSC and 65° C and isolating the DNA polynucleotide detected with SEQ ID NO: 10.

Claim 2: An isolated DNA that hybridizes with SEQ ID NO: 10.

Analysis:

Claim 1:

A review of the full content of the specification indicates that the essential feature of the claimed invention is a process of obtaining a nucleic acid sequence which is identified by a probe that hybridizes to SEQ ID NO:10 and a polynucleotide that hybridizes with SEQ ID NO: 10. The

specification and the general state of the art indicate that the general process of producing nucleic acids through hybridization with probes was routine at the time of filing.

The claim is drawn to a genus i.e., a process of hybridizing to genomic DNA with SEQ ID NO: 10 and isolating the DNA which hybridizes under specific conditions to said sequence.

The search indicates that SEQ ID NO: 10 and SEQ ID NO: 11 are novel and unobvious sequences. Therefore, under the examination guidelines of *In re Ochiai* and *In re Brouwer*, the method of making a novel and unobvious product is also novel and unobvious.

The specification presents an example where a single species has been reduced to practice, i.e., isolation of SEQ ID NO: 11 based on hybridization with SEQ ID NO: 10. Therefore the disclosed species within the genus has been adequately described. Now turning to the genus analysis, the art indicates that there is no substantial variation within the genus because of the stringency of hybridization conditions which yields structurally similar molecules. The single disclosed species is representative of the genus because reduction to practice of this species, considered along with the defined hybridization conditions and the level of skill and knowledge in the art, are sufficient to allow the skilled artisan to recognize that applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus.

Claim 2:

The claim is drawn to a genus of nucleic acids, all of which must hybridize to SEQ ID NO: 10. The claim does not specify any stringency conditions. The claim is broad and reads on virtually any nucleic acid.

There is a species disclosed, SEQ ID NO: 11. The art indicates that there is substantial variation within the genus because the lack of stringency of hybridization conditions would be expected to yield structurally unrelated nucleic acid molecules. The single disclosed species is not representative of the genus because there is no structural attribute or feature that is common to the members of the genus.

Conclusion:

Claim 1 is adequately described.

Claim 2 should be rejected as lacking adequate written description following the analysis described above.

Note: Applicant may overcome the written description rejection of the product by, for example, substituting claim 2 with a product by process claim such as the one below.

Claim 2. *The isolated DNA polynucleotide prepared according to the process of claim 1.*

Example 11: Allelic Variants

Specification: The specification discloses a DNA, SEQ ID NO: 1, said to encode a cell surface receptor for adenovirus. The cell surface receptor is designated protein X and its sequence is given as SEQ ID NO:2. The specification states that the invention includes alleles of the DNA that include single nucleotide polymorphisms (SNPs). No allelic sequence information is disclosed, but the specification states that allelic variants of SEQ ID NO: 1 can be obtained, e.g., by hybridizing SEQ ID NO: 1 to a DNA library made from the species of organism that yielded SEQ ID NO: 1.

Claims:

1. An isolated DNA that encodes protein X (SEQ ID NO: 2).

2. An isolated allele of the DNA according to claim 1, which allele encodes protein X (SEQ ID NO: 2).

3. An isolated allele of SEQ ID NO: 1.

Analysis:

Claim 1:

Claim 1 is drawn to the genus of DNAs that encode amino acid sequence SEQ ID NO:2, i.e., all sequences degenerately related by a genetic code table to SEQ ID NO:1. Although only one specie within the genus is disclosed, SEQ ID NO:1, a person of skill in the art could readily envision all the DNAs degenerate to SEQ ID NO:1 by using a genetic code table. One of skill in the art would conclude that applicant was in possession of the

genus based on the specification and the general knowledge in the art concerning a genetic coding table.

Claim 2:

Claim 2 is drawn to a subgenus of allelic DNAs that encode amino acid sequence SEQ ID NO: 2. The specification does not provide any particular definition for the term allele. In this circumstance, the meaning of the term is the ordinary usage in the art. The ordinary meaning of the term allele is one of two or more alternate forms of a gene occupying the same locus in a particular chromosome or linkage structure and differing from other alleles of the locus at one or more mutational sites. See, Rieger et al., *Glossary of Genetics* (1991), p. 16. The alleles in claim 2 are "strictly neutral" because they encode identical proteins, and make no difference to phenotype. See, Rieger et al., p. 17. Although the standard definition refers to genomic sequences and the claims are directed to DNAs, a reasonable interpretation is that the claim is directed to DNAs that include naturally occurring mutational site(s).

The specification discloses only one allele within the scope of the genus: SEQ ID NO:1. The specification proposes to discover other members of the genus by using a hybridization procedure. There is no description of the mutational sites that exist in nature, and there is no description of how the structure of SEQ ID NO: 1 relates to the structure of any strictly neutral alleles. The general knowledge in the art concerning alleles does not provide any indication of how the structure of one allele is representative of unknown alleles. The nature of alleles is that they are variant structures, and in the present state of the art the structure of one does

not provide guidance to the structure of others. The common attributes of the genus are not described. One of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only one member of this genus is not representative of the variants of the genus and is insufficient to support the claim.

Claim 3:

Claim 3 is drawn to the genus including all DNA alleles of SEQ ID NO: 1. The specification does not provide any particular definition for the term allele. In this circumstance, the meaning of the term is the ordinary usage in the art. The ordinary meaning of the term allele is one of two or more alternate forms of a gene occupying the same locus in a particular chromosome or linkage structure and differing from other alleles of the locus at one or more mutational sites. See, Rieger et al., *Glossary of Genetics* (1991), p. 16. The Rieger reference discloses that there are at least seven different kinds of allele in addition to the "strictly neutral" type discussed above for Claim 2. See, Rieger, pp. 16-17 (amorphs, hypomorphs, hypermorphs, antimorphs, neomorphs, isoalleles, and unstable alleles). The alleles are distinguished by the effect their different structures have on phenotype. According to Rieger, alleles may differ functionally according to their distinct structures. For example, they may differ in the amount of biological activity the protein product may have, may differ in the amount of protein produced, and may even differ in the kind of activity the protein product will have.

The specification discloses only one allele within the scope of the genus: SEQ ID NO:1. The specification proposes to discover other

members of the genus by using a hybridization procedure. There is no description of the mutational sites that exist in nature, and there is no description of how the structure of SEQ ID NO: 1 relates to the structure of different alleles. In addition, according to the standard definition, the genus includes members that would be expected to have widely divergent functional properties. The general knowledge in the art concerning alleles does not provide any indication of how the structure of one allele is representative of other unknown alleles having concordant or discordant functions. The common attributes of the genus are not described and the identifying attributes of individual alleles, other than SEQ ID NO:1, are not described. The nature of alleles is that they are variant structures where the structure and function of one does not provide guidance to the structure and function of others. According to these facts, one of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only one member of this genus is not representative of the variants of the genus and is insufficient to support the claim.

Conclusions:

Claim 1:

Claim 1 should not be rejected under the written description requirement.

Claim 2:

Claim 2 should be rejected under the written description requirement. An analysis similar to the one set forth above could be used. Since the Office has the burden of presenting evidence to support its position, see

MPEP 2163.04, a reference should be relied on as authority for the Office's interpretation of the claim term "allele."

Claim 3:

Claim 3 should be rejected under the written description requirement. An analysis similar to the one set forth above could be used. Since the Office has the burden of presenting evidence to support its position, see MPEP 2163.04, a reference should be relied on as authority for the Office's interpretation of the claim term "allele."

For the rejections of claims 2 and 3, the Office interpretation of "allele" should be supported by a reference, rather than by taking "notice," because the interpretation is the principle evidence supporting the rejection. See MPEP 2144.03 (For further views on official notice, see *In re Ahlert*, 424 F.2d 1088, 1091 165 USPQ 418, 420 - 421 (CCPA 1970) ("[A]ssertions of technical facts in areas of esoteric technology must always be supported by citation of some reference work" and "allegations concerning specific `knowledge' of the prior art, which might be peculiar to a particular art should also be supported." Furthermore the applicant must be given the opportunity to challenge the correctness of such assertions and allegations. "The facts so noticed serve to `fill the gaps' which might exist in the evidentiary showing" and should not comprise the principle evidence upon which a rejection is based.); see also, *In re Barr*, 444 F.2d 588, 170 USPQ 330 (CCPA 1971) (scientific journal references were not used as a basis for taking judicial notice that controverted phrases were art - recognized because the court was not sure that the meaning of the term at issue was indisputable among reasonable men); *In re Eynde*, 480 F.2d 470, 178 USPQ

470,474 (CCPA 1973) ("The facts constituting the state of the art are normally subject to the possibility of rational disagreement among reasonable men and are not amenable to the taking of [judicial] notice.").)

Example 12: Bioinformatics

Specification: The specification discloses a process for identifying and selecting biological compounds that are present in a biological system in a tissue specific manner. In the disclosed process the expression level of a set of compounds is quantitatively determined in multiple tissues within an organism. The expression level data is then graphically displayed in such a manner that compounds that are differentially expressed are easily identified. An artisan interested in identifying a compound that is expressed at a high level in one tissue and at a different level in a second tissue may easily select compounds that are expressed in a tissue specific manner based on the displayed information. The specification indicates that the compounds to be detected encompass DNA, RNA and proteins as well as metabolites. The specification does not provide any particular examples, but discloses that the expression levels can be determined by any analytical method consistent with the class of compounds being detected. This type of measurement requires actual physical steps.

Claim:

A computer-implemented method of selecting tissue specific compounds, said method comprising the steps of:

(a) analyzing the expression level of compounds in a first and second tissue and obtaining expression level data for each of said compounds;

(b) inputting the expression level data obtained in step a) into a computer;

(c) displaying a first axis corresponding to the expression level of each of said compounds in said first tissue;

(d) displaying a second axis substantially perpendicular to said first axis, said second axis corresponding to the expression level data of each of said compound in said second sample

(e) displaying a mark at a position, wherein said position is selected relative to said first axis in accordance with an expression level of each of said compound in said first sample and relative to said second axis in accordance with the expression of said compound in said second sample; and

(f) selecting a compound of interest based on the position of the mark.

Analysis:

A review of the full content of the specification indicates that obtaining, inputting, and displaying the expression level of compounds is essential to the operation of the claimed invention.

A search of the prior art indicates that obtaining the expression level data of compounds is conventional in the art, and that data display devices and associated support algorithms are well known in the art.

A review of the claim indicates that the claim is drawn to a generic environment for the display of compounds in a tissue specific manner.

Since there is no species claimed or disclosed, the claim is analyzed as a claim drawn to a single embodiment. There is no actual reduction to practice of the claimed invention, or clear depiction of the claimed invention

in detailed drawings. However, reading the specification in light of the knowledge and level of skill in the art, the specification discloses the complete steps of the claimed process. See In re Hayes Microcomputer Products Inc. Patent Litigation, 982 F2d. 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992), where the court stated,

> One skilled in the art would know how to program a microprocessor to perform the necessary steps desired in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains.

In this fact situation, the art is sufficiently developed so as to put one of skill in the art in possession of the complete steps of the process. In other words, one skilled in the relevant art would understand what is intended by the claimed invention and know how to carry it out.

Conclusion: There is adequate written description for what is claimed.

Example 13: Protein Variant

Specification: The specification describes a protein isolated from liver. A working example shows that the isolated protein was sequenced and determined to consist of SEQ ID NO: 3. The isolated protein was additionally characterized as being 65 kD in molecular weight and having tumor necrosis activity. The specification states that the invention provides variants of SEQ ID NO: 3 having one or more amino acid substitutions, deletions, insertions and/or additions. No further description of the variants is provided. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and/or additions are routine in the art. The specification does not define when a protein ceases to be a variant of SEQ ID NO: 3.

Claims:

1. An isolated protein having SEQ ID NO: 3.

2. An isolated variant of the protein of claim 1.

Analysis:

Claim 1:

A search of the prior art indicates that SEQ ID NO: 3 is novel and nonobvious. The claim is directed to a genus of proteins that comprise SEQ ID NO: 3. One member of the genus, SEQ ID NO: 3, is described by a complete structure.

There is relatively little variation among the species within the genus because each member of the genus shares SEQ ID NO: 3 as a necessary common feature. The single disclosed example is representative of the claimed genus because taken in view of the general knowledge in the art, the disclosure is sufficient to show that one of skill in the art would conclude that applicant was in possession of the claimed genus.

Claim 2:

This is a genus claim. According to the specification, the term variant means a protein having one or more amino acid substitutions, deletions, insertions and/or additions made to SEQ ID NO: 3. The specification and claim do not indicate what distinguishing attributes shared by the members of the genus. The specification and claim do not place any limit on the number of amino acid substitutions, deletions, insertions and/or additions that may be made to SEQ ID NO: 3. Thus, the scope of the claim includes numerous structural variants, and the genus is highly variant because a significant number of structural differences between genus members is permitted. Although the specification states that these types of changes are routinely done in the art, the specification and claim do not provide any guidance as to what changes should be made. Structural features that could distinguish compounds in the genus from others in the protein class are missing from the disclosure. No common structural attributes identify the members of the genus. The general knowledge and level of skill in the art do not supplement the omitted description because specific, not general, guidance is what is needed. Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, SEQ ID NO: 3 alone is insufficient to

describe the genus. One of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus. Thus, applicant was not in possession of the claimed genus.

Conclusions:

Claim 1:

The claimed subject matter is adequately described. A rejection under the written description requirement should not be entered.

Claim 2:

The claimed subject matter is not supported by an adequate written description because a representative number of species have not been described. A rejection under the written description requirement, relying on the analysis set out above, should be entered.

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

Example 15: Antisense

Specification: The specification discloses a messenger RNA sequence, SEQ ID NO: 1, which encodes human growth hormone. The specification states that the invention includes antisense molecules that inhibit the production of human growth hormone. The specification describes an art-recognized method of screening for antisense molecules that is called "gene walking." Gene walking is said to involve obtaining antisense oligonucleotides that are complementary to the target sequence.

Claim:

An antisense oligonucleotide complementary to a messenger RNA having SEQ ID NO: 1 and encoding human growth hormone, wherein said oligonucleotide inhibits the production of human growth hormone.

Analysis:

A review of the full content of the specification indicates that the complement of SEQ ID NO: 1 is essential to the operation of the claimed invention. The general knowledge in the art is that any full-length complement of a target mRNA inhibits the function of the mRNA and is therefore an antisense oligonucleotide. Thus, one of skill in the art would view applicant's disclosure of a coding sequence, with the statement that the invention includes antisense oligonucleotides, as an implicit disclosure that the full-length complement of SEQ ID NO: 1 is an antisense oligonucleotide.

It is generally accepted in the art that oligonucleotides complementary to a messenger RNA, including fragments of the full-length complement, have antisense activity when they match accessible regions on the target mRNA. Generally, the closer the complementary fragment is to full length, the greater the likelihood it will have antisense activity. In addition, oligos that retain complementarity to the Shine-Delgarno sequence usually have antisense activity.

The claim is drawn to the genus of antisense molecules that inhibit the production of human growth hormone encoded by SEQ ID NO: 1. There is a single species described with a complete structure, i.e., the full-length complement of SEQ ID NO: 1. In addition to the full-length complement, the genus includes fragments of the complement that retain antisense activity.

The procedures for making oligonucleotide fragments of the SEQ ID NO: 1 complement are conventional, e.g., any specified fragment can be ordered from a commercial synthesizing service. The procedures for screening for antisense activity are also conventional, and the specification describes the assay needed to do gene walking. The experience accumulated in the art with gene walking is that numerous regions of a target are accessible, that these regions are identified routinely, and that antisense oligonucleotides are complementary to these accessible regions. The full-length complement and longer fragments match multiple accessible regions; shorter fragments match fewer accessible regions.

When considering the distinguishing characteristics of the claimed invention, the sequence provided in the specification defines and limits the

structure of any effective antisense molecules. The specification also teaches the functional characteristics of the claimed invention as well as a routine art recognized method of making and screening for the claimed invention. Considering the specification's disclosure of:

(1) the sequence (SEQ ID NO: 1) which defines and limits the structure of any effective antisense molecules such that one skilled in the art would be able to immediately envisage members of the genus embraced by the claim, and

(2) the functional characteristics of the claimed invention as well as a routine art-recognized method of screening for antisense molecules which provide further distinguishing characteristics of the claimed invention, along with

(3) the general level of knowledge and skill in the art,

one skilled in the art would conclude that applicant was in possession of the invention.

Conclusion: The claimed invention is adequately described.

Example 16: Antibodies

Specification: The specification teaches that antigen X has been isolated and is useful for detection of HIV infections. The specification teaches antigen X as purified by gel filtration and provides characterization of the antigen as having a molecular weight of 55 KD. The specification also provides a clear protocol by which antigen X was isolated. The specification contemplates but does not teach in an example antibodies which specifically bind to antigen X and asserts that these antibodies can be used in immunoassays to detect HIV. The general knowledge in the art is such that antibodies are structurally well characterized. It is well known that all mammals produce antibodies and they exist in five isotypes, IgM, IgG, IgD, IgA and IgE. Antibodies contain an effector portion which is the constant region and a variable region that contains the antigen binding sites in the form of complementarity determining regions and the framework regions. The sequences of constant regions as well as the variable regions subgroups (framework regions) from a variety of species are known and published in the art. It is also well known that antibodies can be made against virtually any protein.

Claim: An isolated antibody capable of binding to antigen X.

Analysis:

A review of the full content of the specification indicates that antibodies which bind to antigen X are essential to the operation of the claimed invention. The level of skill and knowledge in the art of antibodies at the time of filing was such that production of antibodies against a well-

characterized antigen was conventional. This is a mature technology where the level of skill is high and advanced.

The claim is directed to any antibody which is capable of binding to antigen X.

A search of the prior art indicates that antigen X is novel and unobvious.

Considering the routine art-recognized method of making antibodies to fully characterized antigens, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature, one of skill in the art would have recognized that the spectrum of antibodies which bind to antigen X were implicitly disclosed as a result of the isolation of antigen X.

Conclusion: The disclosure meets the requirement under 35 USC 112 first paragraph as providing an adequate written description of the claimed invention.

Example 17: Genus-species with widely varying species

Specification: The specification discloses the rat cDNA sequences for proinsulin and pre-proinsulin and a method for determining the corresponding human and other mammalian insulin cDNA sequences. However, the specification does not disclose any actual cDNA sequence other than the rat proinsulin and pre-proinsulin sequence. The specification discloses that one human proinsulin amino acid (but not cDNA) sequence was known at the time of filing. The art recognized that the sequence of human insulin proteins, and therefore also cDNAs, would probably vary among individuals. The specification also discloses that pre-proinsulin is post translationally modified to form proinsulin, and that proinsulin is cleaved to form insulin.

Claims:

Claim 1. An isolated mammalian cDNA encoding insulin.

Claim 2. The isolated cDNA of claim 1 wherein the mammalian cDNA is human.

Analysis: The examiner should analyze claim 2 first because it is drawn to a subgenus of the genus of claim 1.

Claim 2:

A review of the full content of the specification indicates that human cDNA molecules that encode insulin are essential to the operation/function of the invention.

Claim 2 is directed to a genus of human cDNA which encodes insulin.

There is no species of human insulin cDNA disclosed.

Based upon art published after applicant's filing date there is expected to be variation among the species of cDNA which encode human insulin because the sequence of human insulin proteins, and therefore also human insulin cDNAs, would be expected to vary among individuals.

The specification discloses only the sequence of a single human proinsulin protein, and does not disclose any human cDNA sequence at all.

In addition, there is no evidence on the record of a relationship between the structure of rat insulin cDNA and the structure of insulin cDNAs from humans or other mammals that would provide any reliable information about the structure of other insulin cDNAs on the basis of the rat insulin cDNA.

There is no evidence on the record that the disclosed rat cDNA proinsulin sequence had a known structural relationship to the human cDNA sequence, or to other mammalian cDNA sequences; the specification discloses only a single human proinsulin (protein) sequence; the art indicated that human proinsulin proteins were expected to be variable in structure; and there is expected to be variation among human cDNAs that

encode a given human proinsulin. In view of the these considerations, a person of skill in the art would not have viewed the teachings of the specification as sufficient to show that the applicant was in possession of the claimed human cDNA.

Claim 1:

Claim 1 is directed to a genus of mammalian cDNAs which encode insulin. The specification evidences actual reduction to practice of the rat cDNA sequences for proinsulin and preproinsulin, but does not disclose any other cDNA sequences. The art indicates that there is likely to be substantial variation among the species within the genus of cDNAs that encode mammalian insulins because the sequences of the mammalian insulin proteins, and therefore the mammalian cDNAs, would be expected to vary among species.

The specification discloses a method for determining the corresponding human and other mammalian insulin cDNA sequences as well as the function of the claimed sequences. However, neither the specification nor the general knowledge of those skilled in the art provide evidence of any partial structure which would be expected to be common to the members of the genus. Moreover, there is post filing date evidence that indicates that there is a lack of a structural relationship between the rat insulin cDNA sequences and other mammalian insulin cDNA sequences. In view of the above considerations one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus, because rat cDNA sequences are not representative of the claimed genus. Consequently, since applicant was in

possession only of the rat insulin cDNA and since the art recognized variation among the species of the genus of cDNAs that encode mammalian insulin, the rat insulin cDNA was not representative of the claimed genus. Therefore, the applicant was not in possession of the genus of mammalian insulin cDNAs as encompassed by claim 1.

Conclusion:

Claims 1 and 2 do not meet the written description requirement.

Example 18: <u>Process claim where the novelty is in the method steps.</u>

Specification: The specification teaches a method for producing proteins using mitochondria from the fungus *Neurospora crassa.* In the method, mitochondria are isolated from this fungus and transformed with a mitochondrial expression vector which comprises a nucleic acid encoding a protein of interest. The protein is subsequently expressed, the mitochondria is lysed, and the protein is isolated. The specification exemplifies the expression of β-galactosidase using the claimed method using a cytochrome oxidase promoter.

Claim:

1. A method of producing a protein of interest comprising;

obtaining *Neurospora crassa* mitochondria,

transforming said mitochondria with a expression vector comprising a nucleic acid that encodes said protein of interest,

expressing said protein in said mitochondria, and

recovering said protein of interest.

Analysis:

A review of the specification reveals that *Neurospora crassa* mitochondrial gene expression is essential to the function/operation of the claimed invention. A particular nucleic acid is not essential to the claimed invention.

A search of the prior art reveals that the claimed method of expression in *Neurospora crassa* is novel and unobvious.

The claim is drawn to a genus, i.e., any of a variety of methods that can be used for expressing protein in the mitochondria.

There is actual reduction to practice of a single embodiment, i.e., the expression of β-galactosidase.

The art indicates that there is no substantial variation within the genus because there are a limited number of ways to practice the process steps of the claimed invention.

The single embodiment is representative of the genus based on the disclosure of *Neurospora crassa* mitochondria as a gene expression system, considered along with the level of skill and knowledge in the gene expression art. One of skill in the art would recognize that applicant was in possession of all of the various expression methods necessary to practice the claimed invention.

Conclusion:

The claimed invention is adequately described.